ZOOLOGIA 36: e30354
ISSN 1984-4689 (online)
zoologia.pensoft.net

RESEARCH ARTICLE

Does the widely distributed rodent *Calomys tener* (Cricetidae: Sigmodontinae) constitute a single evolutionary unit?

Raul E. González-Ittig[1], Narayan P. Kandel[2], Cibele R. Bonvicino[3], Jorge Salazar-Bravo[2]

[1]*Instituto de Diversidad y Ecología Animal, CONICET, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba. Avenida Vélez Sársfield 299, 5000JJC Córdoba, Argentina.*
[2]*Department of Biological Sciences, Texas Tech University. PO Box 43131, 79409 Lubbock, Texas, USA.*
[3]*Divisão de Genética, Instituto Nacional de Câncer. Rua André Cavalcanti 37, 4º andar, 20231-050 Rio de Janeiro, RJ, Brazil.*
Corresponding autor: Raúl E. González-Ittig (raulgonzalezittig@yahoo.com.ar)

http://zoobank.org/27DD8BCE-FE46-4234-9202-619A11DB3582

ABSTRACT. The nominal species *Calomys tener* (Winge, 1887) ranges broadly in open lands of the Caatinga, Cerrado, Pantanal and Mata Atlântica of Brazil, and was recently reported from the Pampas of southern Brazil, and in the Selva Paranaense of eastern Paraguay and northeastern Argentina. This rodent can be infected with the pathogenic Araraquara hantavirus in Brazil. Given that most epidemiological studies have not taken into account updated taxonomic findings of their rodent hosts, in this study, we obtained sequence data of the Cyt-b and COI genes of specimens of *C. tener* from 22 different geographical localities from throughout the currently known distribution of the species (including individuals from Argentina, Paraguay, Bolivia, and Brazil) to test if it constitutes a single genetic unit or if it presents genetic discontinuities that may represent different evolutionary lineages. Phylogenetic analyses including several species of *Calomys* recovered several clades with strong support. Regarding *C. tener*, it is recovered as sister to the node that cluster *C. laucha* (Fischer, 1814) *sensu lato*, *C. expulsus* (Lund, 1841) and species in the *C. callosus* (Rengger, 1830) species complex. At the intraspecific level there are no genetic gaps among haplotypes of *C. tener* that could suggest more than one species. The recent captures in the Pampas of southern Brazil and in the Selva Paranaense suggest that the species may be colonizing new geographic areas.

KEY WORDS. Cyt-b, phylogenetic relationships, South America.

INTRODUCTION

Geographic ranges are dynamic properties of species, easily modified in response to biotic and abiotic drivers (Case et al. 2005, Soberon 2007, Linder et al. 2012, Simonov 2014). New locality records, new country records, or new habitat records ought to be documented as they may indicate range extensions resulting from demographic expansions and/or deficiency in species geographical samplings. Cases like these have been observed for example in *Loxodontomys micropus* (Waterhouse, 1837) (see Cañon et al. 2010), in *Dromiciops gliroides* Thomas, 1894 (see D'Elia et al. 2016, but also see Suárez-Villota et al. 2018), among many others.

Despite a renewed interest in the taxonomy and systematics of South American sigmodontine rodents, many taxa still lack detailed analyses of morphological, geographical, genetic and ecological variation; thus, the alpha-taxonomy of these groups is only partially resolved. Detailed morphological and genetic studies provide insights into the biology and evolution of species with large anthropogenic impact, as is the case of species of zoonotic or agriculture concern (for example, Yackulic et al. 2010, Pigot and Tobias 2013, Bordes et al. 2015). An example of this is the genus *Calomys* Waterhouse, 1837 where several species share similar morphological and morphometric characteristics, many of which are only recently being duly appreciated (Almeida et al. 2007, Haag et al. 2007, Salazar-Bravo 2015). A couple of the species in the genus have received recent interest, for example *Calomys laucha* (Fischer, 1814) (González-Ittig et al. 2014) or *Calomys sorellus* (Thomas, 1900) (Zeballos et al. 2014); in both bases, cryptic species within currently recognized taxonomic units were suggested.

One species of the genus that needs a study of its taxonomic situation is *Calomys tener* (Winge, 1887), the Delicate Vesper Mouse, a small body-sized species, broadly distributed in open vegetative formations in the Cerrado of central Brazil and eastern Bolivia, Caatinga and Pantanal in Brazil (Salazar-Bravo

2015). Several novel reports of occurrence for *C. tener* have been published: for example, the species was recently reported in the Atlantic and Paranaense forests of Argentina and Paraguay and areas in southeastern Brazil (González-Ittig et al. 2014, Quintela et al. 2014), all of which were considered outside of the range of the species (Salazar-Bravo 2015). The ecology of *C. tener* is better known in Brazil, where it occupies a mix of habitat types, including domestic and agricultural areas, and populations appear to be stable all year-long (Umetsu and Pardini 2007). Individuals in a handful of populations in São Paulo state are known to be infected with Araraquara hantavirus, currently considered the most pathogenic of all South American hantaviruses (Figueiredo et al. 2010). It has been estimated that more than 50% of total hantavirus pulmonary syndrome (HPS) cases in Brazil are caused by Araraquara hantavirus and that the human fatality rate is over 40% for infected patients (Figueiredo et al. 2009). Although *Necromys lasiurus* (Lund, 1840) is the natural reservoir of this viral genotype, the role of *C. tener* in the circulation of the virus is not completely understood; however, reports of the occurrence of this species in other areas of Brazil and elsewhere in South America should be considered an element of risk for the potential presence of this pathogenic virus in these areas. As suggested by Mills and Childs (1998) and Mills et al. (1999), reservoir studies, including those related to their systematics, taxonomy, and geographic distribution, are necessary for a comprehensive understanding of the biology of emerging zoonotic diseases.

In the case of *C. tener*, different molecular systematic studies have included sequences from few localities (see Table 1). For example, Salazar-Bravo et al. (2001) included sequences of the mitochondrial cytochrome b (Cyt-b) gene from individuals of one locality in Bolivia and one in Brazil. Almeida et al. (2007) used the sequences of Salazar-Bravo et al. (2001) and added Cyt-b data of individuals from five sites in Brazil. Haag et al. (2007) also used the sequences obtained by Salazar-Bravo et al. (2001) and added molecular information of the same gene from individuals from three extra localities in Brazil. González-Ittig et al. (2014) in a study focused on the systematics of *C. laucha*, identified three individuals from Paraguay and Argentina that actually corresponded to *C. tener*. The results of all these studies have recovered *C. tener* as monophyletic without any phylogenetic structure or sub-clades, however, these conclusions are partial since none have integrated the whole information available for the species. To detect possible genetic gaps among specimens of this species over a broad geographic area, in the present study we compared sequences of the mitochondrial genes Cyt-b and cytochrome oxidase subunit 1 (COI) of individuals from Argentina, Paraguay, Bolivia, and Brazil, encompassing most of the known distribution of the rodent.

MATERIAL AND METHODS

Partial sequences of the Cyt-b (788bp) of 31 individuals identified as *C. tener* were used for this study: from Brazil (n = 19), Bolivia (n = 1), Paraguay (n = 4), and Argentina (n = 7) and five partial sequences of the COI from Brazil, giving a total of 22 sampled localities (Fig. 1, Table 1). The five sequences of the COI gene were published by Müller et al. (2013). Of the Cyt-b sequences, 19 were published in different studies listed in Table 1, whereas 12 were generated at the lab of Jorge Salazar-Bravo (Texas Tech University, Lubbock, Texas, USA) from samples obtained on loan from several institutions (see Acknowledgements).

Tissues were processed for DNA extraction using DNeasy Blood and Tissue kit (Qiagen, Cat# 69505) and the manufacturer's recommendations. DNA was quantified using Nanodrop (NanoDrop™ 1000 Spectrophotometer v3.7) and 1% agarose gels. A fragment of the mitochondrial Cyt-b gene was amplified using one of two primer combinations of Mus14095-F and Mus15398-R or L14415-F and Mus15398-R (González-Ittig et al. 2014). Thermocycler conditions were: initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of denaturation at 94 °C for 45 seconds, annealing at 50 °C for 1 minute, and extension at 72 °C for 1.5 minutes, and final extension at 72 °C for 5 minutes. Reaction mixtures were set at 25µL total volume with 1 µL of DNA (20–30 ng/µL), 0.13 µL of Promega Taq (Cat# M3008, 5U/µL), 0.8 µL of dNTPs mix (Cat#U151B, 10mM), 2 µL of $MgCL_2$ (25 mM), 2.5 µL of 5 × Go Taq Green buffer, and 0.75 µL of each primer (10 µM). Amplified products were visualized in 1% agarose gel; 1Kb ladder was used as standard DNA size. Well amplified products were sequenced in Macrogen, USA (http://www.macrogenusa.com) with both amplifying primers. Raw chromatograms were cleaned, translated to amino-acids to check for premature stop codons and other nonfunctional mutations. Contigs for individual sequences were assembled using SeqManII 5.07 (Lasergene, DNASTAR, Madison, WI, USA). New sequences of *C. tener* were deposited in GenBank (Table 1).

All sequences, including those available online, were aligned using default parameters with ClustalW in MEGA5.0 (Tamura et al. 2011). Identical sequences of *C. tener* were identified and collapsed into haplotypes using PopART v1.7 (Leigh and Bryant 2015). Haplotypes were used for all further phylogenetic analyses; in the matrix we included sequences published by Almeida et al. (2007), Haag et al. (2007) and González-Ittig et al. (2014) corresponding to the following species: *Calomys expulsus* (Lund, 1841), *C. fecundus* (Thomas, 1926), *C. callosus* (Rengger, 1830), *C. hummelincki* (Husson, 1960), *C. lepidus* (Thomas, 1884), *C. venustus* (Thomas, 1894), *C. musculinus* (Thomas, 1913), *C. tocantinsi* Bonvicino, Lima & Almeida, 2003, *C. sorellus* and *C. laucha* (both clades A and B). Sequences of *Eligmodontia typus* Cuvier, 1837, *Auliscomys sublimis* (Thomas, 1900), *Phyllotis xanthopygus* (Waterhouse, 1837), *Andalgalomys pearsoni* (Myers, 1977) and *Salinomys delicatus* Braun & Mares, 1995 were used as outgroups. Maximum Parsimony (MP) analysis was performed with PAUP 4.0.b10 (Swofford 1998), where characters were unordered and equally weighted. We performed a heuristic search of 1000 iterations of random taxon addition using the TBR (tree bisection-reconnection) branch swapping algorithm. Maximum Parsimony

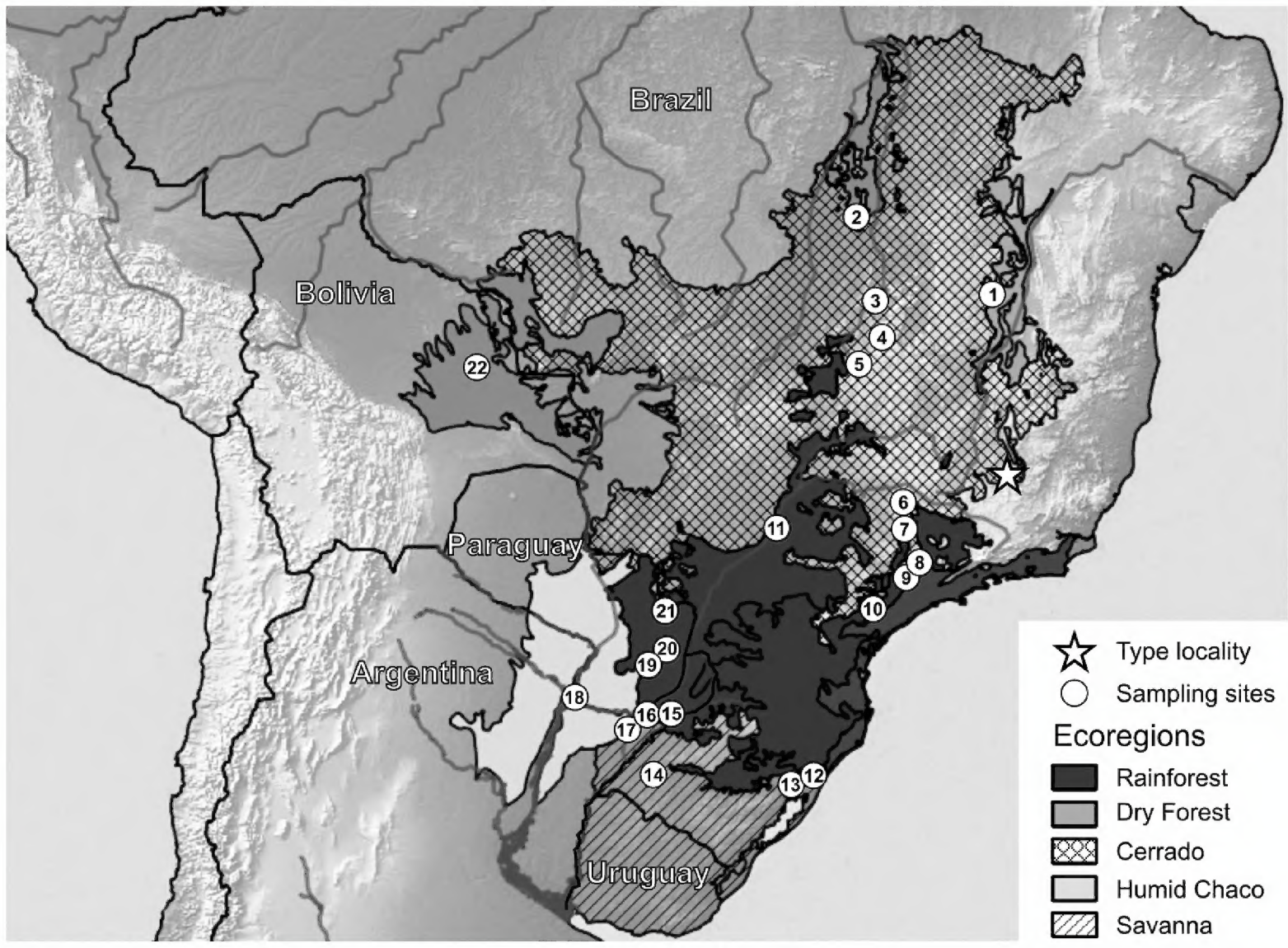

Figure 1. Geographical locations of individuals listed in Table 1. Vegetation formations are represented by ecoregions like Cerrado, Humid Chaco, Rainforests (including the phytogeographic regions Alto Paraná Atlantic forest + Araucaria moist forest + Serra do Mar coastal forest), Dry Forests (Mato Grosso seasonal forest + Atlantic dry forest) and Savannas (Southern cone Mesopotamian savanna + Uruguayan savanna). The star indicates the type locality of *Calomys tener*: Lagoa Santa in the state of Minas Gerais corresponding to the Cerrado ecoregion. This map was designed using free spatial data of QGIS (Quantum GIS Development Team, 2018), available at: http://qgis.osgeo.org.

analyses resulting in more than one most parsimonious tree were summarized in a strict consensus tree. Nonparametric bootstrap support values were calculated using 1000 replicate searches. The best-fitting model of sequence evolution was selected using jModeltest 2 (Darriba et al. 2012) in which likelihood scores for 88 different models were computed. The HKY+I+G model was selected using the Bayesian information criterion and the following starting parameters were used subsequently in Bayesian inferences (BI) and Maximum Likelihood (ML) analyses: a base frequency of A = 0.3030, C = 0.3096, G = 0.0877, T = 0.2997; a transition/transversion = 4.233; a proportion of invariable sites of 0.4860; a gamma distribution with alpha = 1.0360. The BI analyses were performed using MrBayes 3.2 (Ronquist et al. 2012) with two independent Markov chain-Monte Carlo (MCMC) runs, with one cold and three heated chains each. Runs were performed for five million generations and trees were sampled every 1000 generations. We discarded the first 25% of the samples as "burn in" and the two runs converged on similar posterior estimates with an average standard deviation of split frequencies of 0.006. We assessed convergence and mixing visually using Tracer v1.5 to plot likelihood scores for all parameters by generation time and by calculating effective sample sizes (Rambaut and Drummond 2007). ML trees were constructed using the online version of program PhyML ver 3.0 (http://www.atgc-montpellier.fr/phyml) (Guindon et al. 2010). In PhyML we used the same substitution model and the parameters described above; 1000 bootstrap replicates were performed.

In addition, a median-joining network was constructed using PopART v1.7 with 1,000 permutations: the method estimates the relative abundance of each haplotype and the genealogical relationships among them. Kimura-2 parameter genetic distances (K2p) among haplotypes of *C. tener* were calculated with MEGA5.0.

RESULTS

A total of 28 distinct haplotypes were identified from the 31 *C. tener* individuals analyzed. In the alignment with other species of *Calomys* and outgroups of other genera, from the 794 sites, 342 characters were variable of which 275 were parsimony-informative. In the phylogenetic trees obtained with different phylogenetic methods (ML, BI and MP) for species of

Table 1. Field identification code or voucher numbers of specimens of the Delicate Vesper Mouse (*Calomys tener*). The location of the sampling sites shown in Fig. 1 with provinces or departments in parentheses; geographic coordinates; Cyt-b GenBank accession numbers (there are five accession numbers corresponding to the cytochrome oxidase I gene indicated in parenthesis with COI) and articles were the sequences were obtained.

Specimen ID	Sample Location	Latitude	Longitude	GenBank registers	Haplotype	Reference
	Brazil					
CRB1549	1- Jaborandi (Bahia)	-13.6188	-44.4328	DQ447298	20	Almeida et al. (2007)
CRB1558	1- Jaborandi (Bahia)	-13.6188	-44.4328	DQ447299	21	Almeida et al. (2007)
CRB1584	1- Jaborandi (Bahia)	-13.6188	-44.4328	DQ447300	22	Almeida et al. (2007)
CRB1590	1- Jaborandi (Bahia)	-13.6188	-44.4328	DQ447301	23	Almeida et al. (2007)
AN2557	2- Aliança do Tocantins (Tocantins)	-11.0050	-48.9333	AY964055	12	Haag et al. (2007)
MN36437	3- Serra da Mesa (Goiás)	-13.8341	-48.3044	AY964053	10	Haag et al. (2007)
CRB2382	4- Mimoso de Goiás (Goiás)	-15.0483	-48.0833	DQ447302	24	Almeida et al. (2007)
MNRJ67075/CRB2382	4- Mimoso de Goiás (Goiás)	-15.0483	-48.0833	GU938935 (COI)	A	Muller et al. (2013)
CRB503	5- Corumbá de Goiás (Goiás)	-15.9241	-48.8085	DQ447295	17	Almeida et al. (2007)
CRB495	5- Corumbá de Goiás (Goiás)	-15.9241	-48.8085	KX987844	18	This study
FMRP-USP96	6- Franca (São Paulo)	-20.5333	-47.4000	KX987845	15	This study
CPV425	7- Cajuru (São Paulo)	-21.2540	-47.3104	KX987842	13	This study
CPV432	7- Cajuru (São Paulo)	-21.2540	-47.3104	KX987843	14	This study
CRB1219	8- Pedreira (São Paulo)	-22.7419	-46.9014	DQ447296	13	Almeida et al. (2007)
CRB1220	8- Pedreira (São Paulo)	-22.7419	-46.9014	DQ447297	19	Almeida et al. (2007)
EM1135	9- Campinas (São Paulo)	-22.9008	-47.0572	DQ447294	16	Almeida et al. (2007)
LDCM-AB6	10- Capão Bonito (São Paulo)	-24.0600	-48.3200	GU939000 (COI)	C	Muller et al. (2013)
LDCM-AB519	10- Capão Bonito (São Paulo)	-24.0600	-48.3200	GU939001 (COI)	C	Muller et al. (2013)
LDCM-AB10	10- Capão Bonito (São Paulo)	-24.0600	-48.3200	GU939002 (COI)	B	Muller et al. (2013)
NK42183	11- Tupi Paulista (São Paulo)	-21.3873	-51.5678	AF385597	9	Salazar-Bravo et al. (2001)
NK42140	11- Tupi Paulista (São Paulo)	-21.3873	-51.5678	AF385596	8	Salazar-Bravo et al. (2001)
MCN-MAM42	12- Banhado Grande (Rio Grande do Sul)	-30.0117	-50.9650	JX975467	25	Quintela et al. 2014
AFV02	13- Quintão (Rio Grande do Sul)	-29.6666	-50.2666	AY964054	11	Haag et al. (2007)
UFPB7407/JR405	14- Alegrete (Rio Grande do Sul)	-29.5700	-55.7100	GU938944 (COI)	D	Muller et al. (2013)
	Argentina					
34769	15- Leandro N Alem (Misiones)	-27.5833	-55.2667	KF917370	6	González-Ittig et al. (2014)
LTU687	16- Estancia Santa Inés (Misiones)	-27.5256	-55.8719	KX987847	2	This study
LTU692	16- Estancia Santa Inés (Misiones)	-27.5256	-55.8719	KX987848	3	This study
LTU711	16- Estancia Santa Inés (Misiones)	-27.5256	-55.8719	KX987849	4	This study
LTU726	16- Estancia Santa Inés (Misiones)	-27.5256	-55.8719	KX987850	5	This study
LTU341	17- RN 12 y Arroyo Itaembé Miní (Misiones)	-27.4300	-56.0000	KX987856	1	This study
LTU342	17- RN 12 y Arroyo Itaembé Miní (Misiones)	-27.4300	-56.0000	KX987846	1	This study
	Paraguay					
TK66069	18- Estancia San Felipe, 2km S house (Ñeembucú)	-27.1833	-58.3833	KF917377	28	González-Ittig et al. (2014)
TK63733	19- Reserva Natural Privada Ypeti (Caazapá)	-25.6825	-55.5300	KX987852	26	This study
TK63769	20- Estancia Golondrina (Caazapá)	-25.6010	-55.4865	KX987853	27	This study
TK63984	21- Res. Nat. del Bosque Mbaracayu (Canindeyú)	-24.1601	-55.2832	KF917376	12	González-Ittig et al. (2014)
	Bolivia					
NK21054	22- Santa Rosa de La Roca (Santa Cruz)	-16.0500	-61.5667	AF385595	7	Salazar-Bravo et al. (2001)

Calomys, several clades are identified with strong support. For example, the, one composed by the species *C. lepidus/C. sorellus/C. musculinus*. The phylogenetic position of *C. hummelincki* is not resolved, since the node has low support values. Another clade strongly supported is the one composed by the species *C. expulsus* and species of the *C. callosus* complex (*C. tocantinsi/C. callosus/C. fecundus/C. venustus*) (Fig. 2). Sister to this clade is the one composed by clades A and B of *C. laucha* (*sensu* González-Ittig et al. 2014). The species *C. tener* presents a sister position in relation to the node that clusters *C. laucha sensu lato*, *C. expulsus* and *C. callosus sensu lato*.

At the intraspecific level there is no phylogenetic structure among haplotypes of *C. tener* that would suggest more than one species, even when samples have been collected throughout a large part of its geographical distribution (encompassing individuals from Argentina, Paraguay, Bolivia, and Brazil). In Fig. 2, haplotypes from localities 1, 4, 5, 12 and 13 situated in Brazil are the first to split off, while those from localities 15, 16 and 17 located in Argentina are among the last. The median-joining network (Fig. 3) showing the frequencies and the relationships of the 28 haplotypes does not corroborate these ancestral-derived positions, since none of the haplotypes 11, 17, 18, 20, 21, 22,

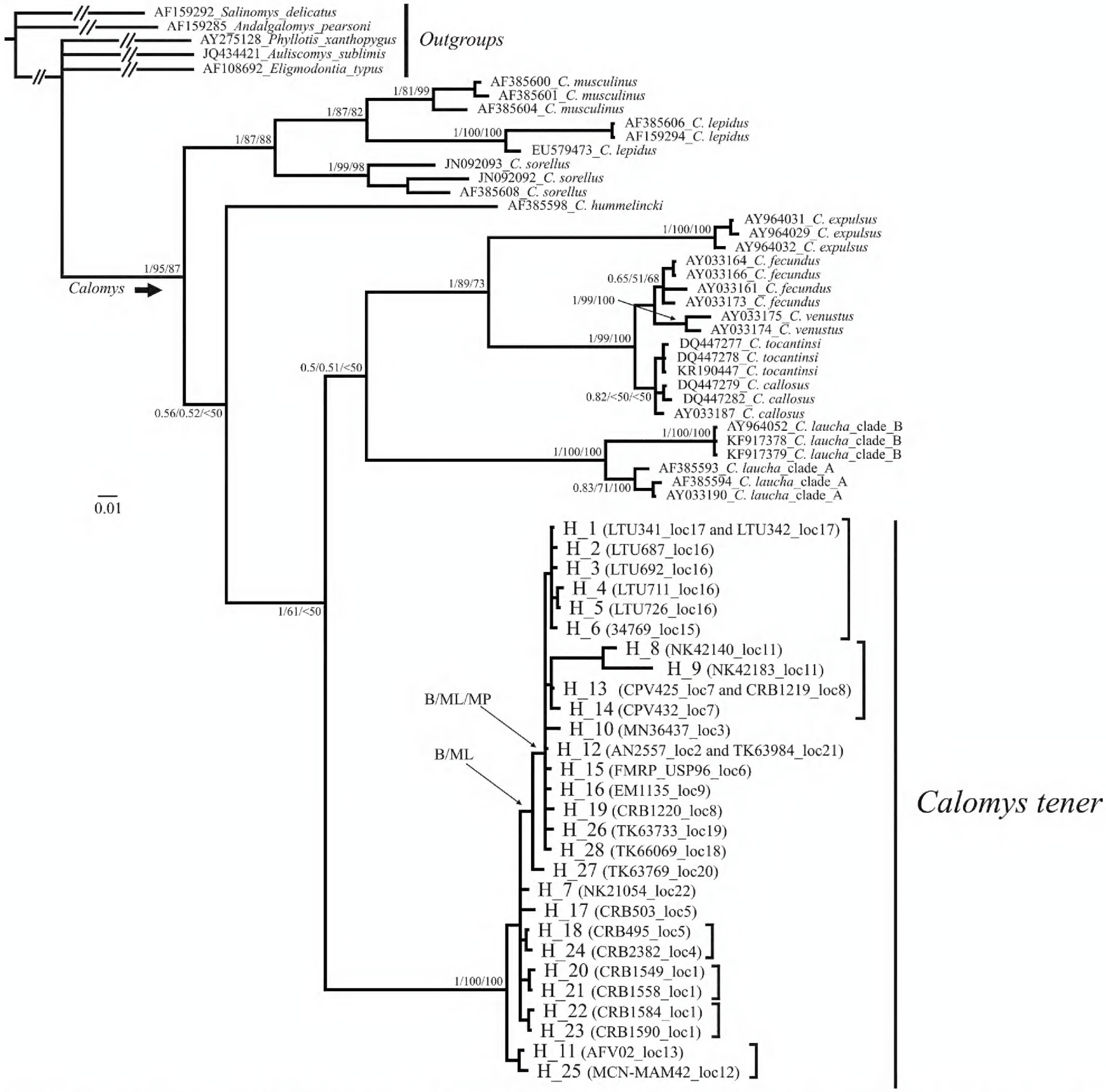

Figure 2. Phylogram of the Bayesian consensus tree obtained from Cyt-b data set for species of *Calomys* with emphasis in *Calomys tener*. The order of the support values in the nodes is as follows: Bayesian posterior probabilities/maximum likelihood bootstrap/maximum parsimony bootstrap. Sequences from GenBank are indicated with their accession number and the species names. In the clade corresponding to *C. tener* the haplotype number, the specimen having each haplotype and the sampling locality is indicated. The vertical bars represent associations also observed in the network of Fig. 3, although with no statistical support. The intraspecific relationships recovered with the different phylogenetic methods are indicated.

23, 24 or 25 are in the center of the network. No obvious pattern was observed between haplotype identity and geographic locations, except for a tendency that haplotypes unique to Argentina (haplotypes 1, 2, 3, 4, 5 and 6) group together on one side of the network and the Brazilian haplotypes mentioned above are in the other side of the network (Fig. 3). Although with low support, some relationships among haplotypes in Fig. 3 are also recovered with the three or with two different

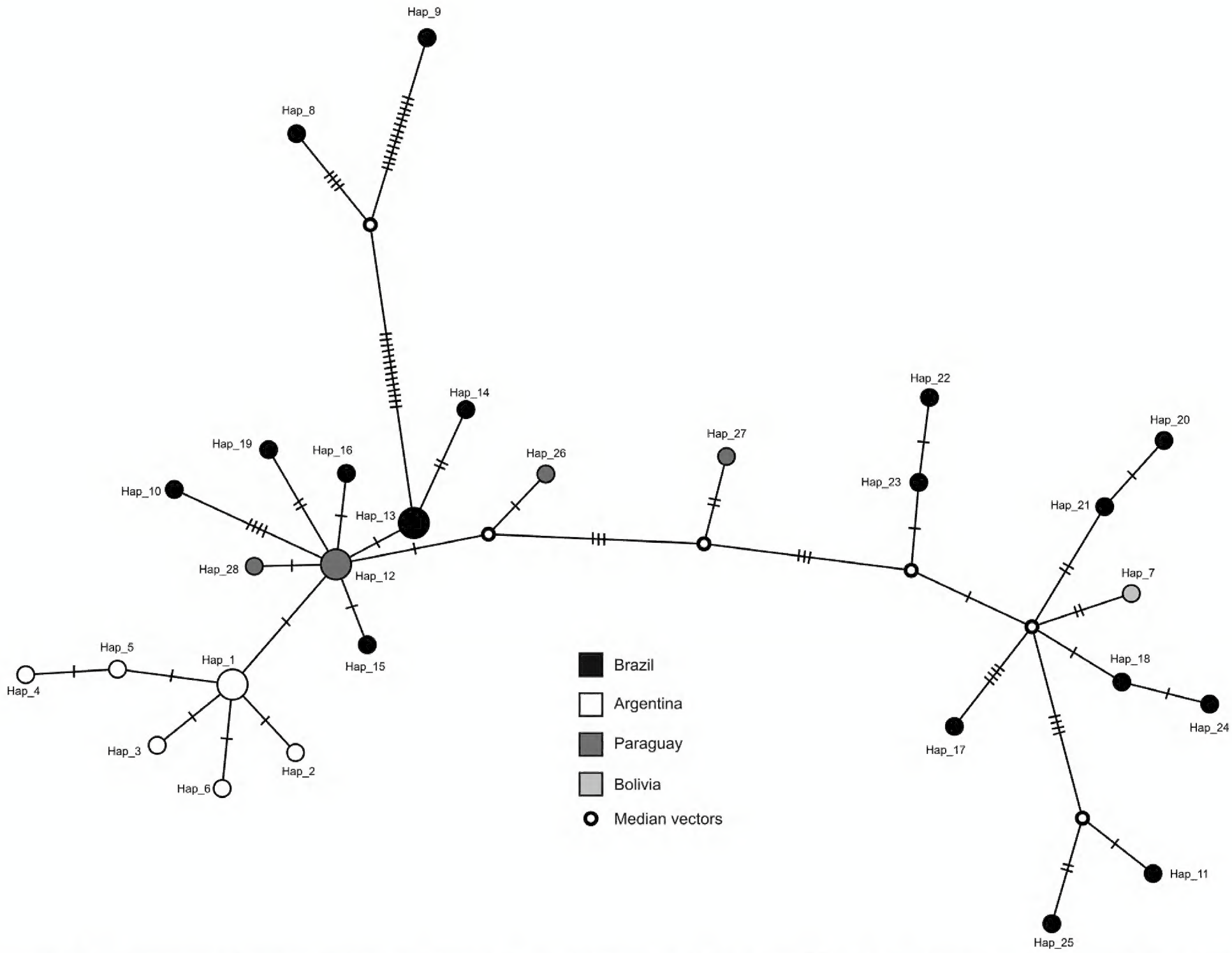

Figure 3. Median-joining network showing the relationships and relative abundance of the 28 haplotypes (indicated by a number) detected in *Calomys tener*. The different colors indicate the country where each haplotype was detected. Each bar through the solid line represents one nucleotide difference between haplotypes. The black circle (mv1) represents a median vector.

phylogenetic methods (indicated in Fig. 2 with a vertical bar). The K2p genetic distances among *C. tener* haplotypes averaged 1.29% ± 1.05, also compatible with intraspecific genetic variation (Bradley and Baker 2001). In the network, haplotypes 8 and 9 from locality 11 are separated from the remaining haplotypes by more than 12 mutations, moving away from the general trend of diversification within *C. tener*.

Four CO1 haplotypes were identified among the five individuals of *C. tener* sequences. In the alignment with other species of *Calomys* and outgroups, from the 640 sites, 173 characters were variable of which 100 were parsimony-informative. The phylogenetic trees obtained ML, BI and MP have the same topology with varying levels of support for each node. The clade of *C. tener* is strongly supported and has a sister relationship with a clade formed by *Calomys cerqueirai* Bonvicino, Oliveira & Gentile, 2010 and *C. expulsus* (Fig. 4). As in the Cyt-b dataset, there are no large genetic gaps among haplotypes of *C. tener* that could suggest more than one species, even though samples were collected in distantly locations from Brazil (locations 4, 10 and 14 in Fig. 1). In addition, the K2p genetic distances among *C. tener* haplotypes averaged 1.31 ± 1.01%.

DISCUSSION

In this study, we used the Cyt-b gene from 31 individuals and the COI gene from five individuals collected throughout the range of the Delicate Vesper Mouse. Our sampling was broad and includes the known distribution range of the species as currently understood (Salazar-Bravo 2015). The mean genetic distance detected was 1.29% and 1.31% with the Cyt-b and COI genes, respectively, which are in line with the estimations

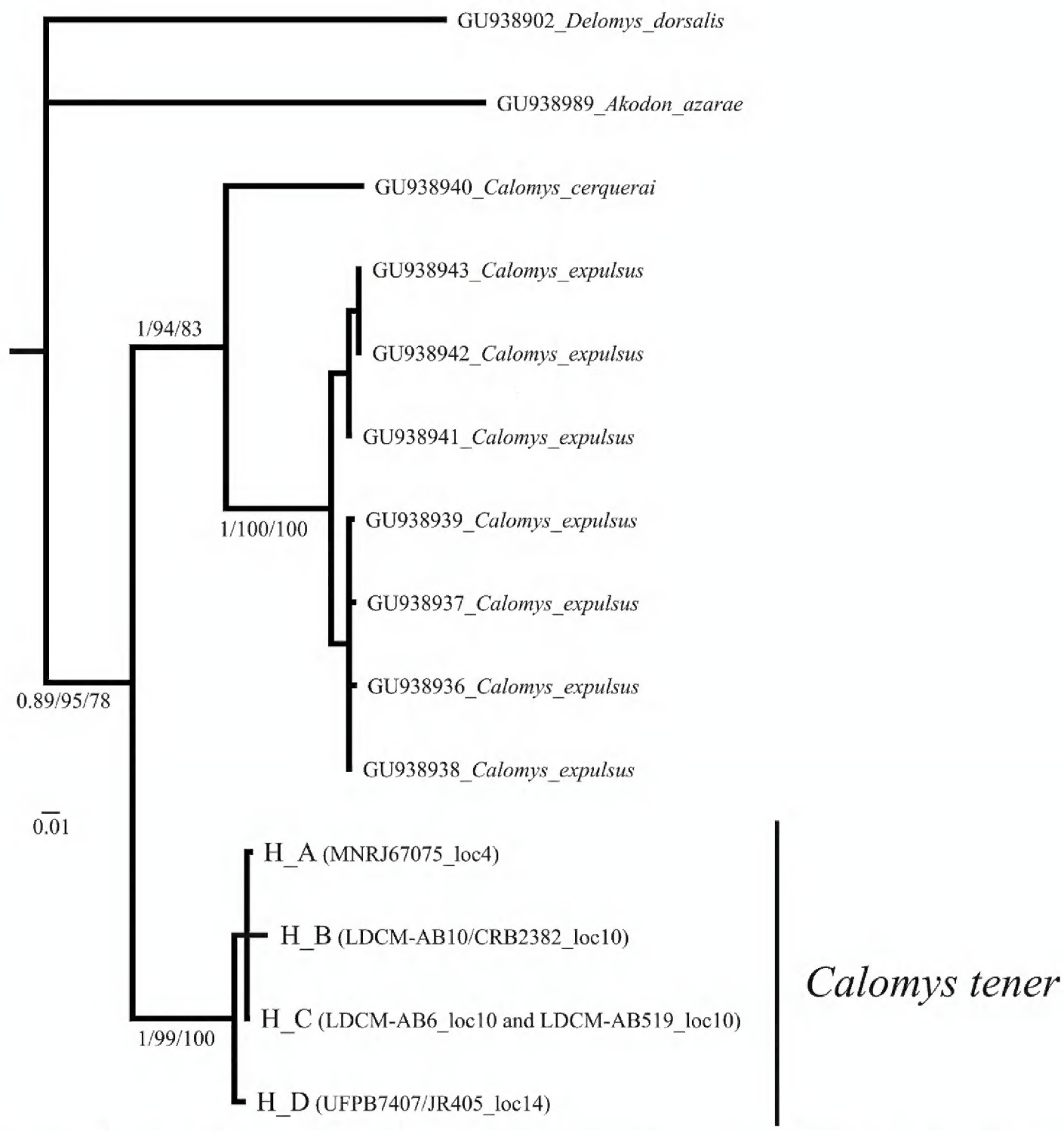

Figure 4. Phylogram of the Bayesian consensus tree obtained from COI data set for species of *Calomys* with emphasis in *Calomys tener*. The order of the support values in the nodes is as follows: Bayesian posterior probabilities/maximum likelihood bootstrap/maximum parsimony bootstrap. Sequences from GenBank are indicated with their accession number and the species names. In the clade corresponding to *C. tener* the haplotype letter, the specimen having each haplotype and the sampling locality is indicated.

for intraspecific comparisons according to Bradley and Baker (2001). These estimates are also in accordance with intraspecific variation reported for other species of *Calomys* (e.g., Salazar-Bravo et al. 2001, Almeida et al. 2007, González-Ittig et al. 2007, Bonvicino et al. 2010, Nascimento et al. 2011). It is important to highlight that the monophyletic nature of *C. tener* was supported with two markers (Figs 2, 4). Notwithstanding, this study was performed only with mitochondrial DNA which does not undergo recombination, has uniparental inheritance, and reflects only the female evolutionary history. The shortcomings of this approach are evident when males and females have different dispersal patterns or when closely related species hybridize, so the resulting genetic structure could potentially be different for both sexes. Therefore, to confirm that *C. tener* has low levels of intraspecific divergence and to reflect the diversification processes of both sexes, nuclear genes should be included in further studies (Dupuis et al. 2012).

The taxonomic uniformity of the species was already suggested by cytogenetic studies performed by other authors over the last 30 years. For example, Mattevi et al. (2005) reported 2n = 66 (FN = 66–68) for specimens from Minaçú (Goiás). A 2n = 66 (FN = 66) was reported by Bonvicino et al. (2010) for individuals from Santa Tereza (Espírito Santo), Jaborandi (Bahia), Mimoso de Goiás (Goiás) (specifically the Cyt-b of individual MN36437, karyotyped in that study, was included in the present study, Table 1). This chromosome number was also reported for specimens from Distrito Federal, Cocos (Bahia), Campinas, Itirapina, Itapetininga, Pedreira and Rio Claro, all in

São Paulo state and Gaúcha do Norte (Mato Grosso) (Yonenaga 1975, Bonvicino and Almeida 2000, Fagundes et al. 2001). In addition, Haag et al. (2007) detected the chromosomal number 2n = 66 (FN = 66–70) for specimens from Serra da Mesa (Goiás), Aliança do Tocantins (Tocantins), Quintão (Rio Grande do Sul), Tupi Paulista (São Paulo) all in Brazil and one specimen from Santa Rosa de la Roca (Santa Cruz) in Bolivia. Haag et al. (2007) also sequenced the Cyt-b of all the karyotyped specimens which were also included in the present study (Table 1).

In this study, the only discordant result that alters genetic continuity, is that involving haplotypes 8 and 9 from locality 11, which are very divergent. Given the central geographic position of this locality (Fig. 1) and the general level of genetic differentiation within the species, these haplotypes could be the result of sequencing errors as was already advocated by Almeida et al. (2007). Therefore, the molecular results obtained here and the substantial cytogenetic evidence, support the inference that *C. tener* constitutes only one taxonomic unit. The type locality of the species is Lagoa Santa in the state of Minas Gerais in Central Brazil corresponding to the Cerrado ecoregion. Notwithstanding, it should be noted that Mattevi et al. (2005) reported the chromosomal complement for two specimens (LF4974 and LF5020) from Rondônia state, Brazil, with 2n = 64 (FN = 64) and suggested they could correspond to *C. tener*. However, the authors, with caution, denominated these individuals as *Calomys* sp. Given the chromosomal uniformity detected by many authors for *C. tener*, it is unlikely that the karyotype from Rondônia corresponds to this species because it lacks one autosomal pair and presents differences in the size and shape of the X chromosome. Unfortunately, no molecular data is available for the two specimens from Rondônia, therefore we cannot explicitly test the hypothesis that either of these correspond to *C. tener*.

The taxonomic status of *C. tener* and *C. laucha* are historically intertwined; originally described in the late 19th century, *C. tener* maintained its specific status until Philip Hershkovitz included it in his polytypic concept of *C. laucha* (Hershkovitz 1962). The status of *C. tener*, with respect to *C. expulsus* and *C. callosus* was forcefully established by Bonvicino and Almeida (2000), but it was not until Salazar-Bravo et al. (2001) that a *C. laucha* and *C. tener* were included in a phylogenetic analysis of the genus. In this work, Salazar-Bravo et al. (2001) resolved these species as sister taxa, with little or no support. Further work by Almeida et al. (2007) showed –with a larger sampling of taxa and individuals – that *C. tener* was in fact the sister taxon to all lowland forms of *Calomys*. Similar results were reported by Bonvicino et al. (2010). A great part of the current taxonomic confusion stems from the similar morphological and morphometric characteristics of *C. tener* and *C. laucha*. In fact, they are so similar morphologically that even the last reviser of the genus confused individuals of *C. tener* from Mato Grosso for *C. laucha* (Olds 1988). Although *C. tener* is more or less uniform across its geographic range (upper parts of body yellowish to dark brown, with a reddish hue in most specimens; ventral region grayish to whitish with base of hairs gray) the geographical variation in *C. laucha* is extensive (as described in Salazar-Bravo 2015). In fact, in areas of potential geographical overlap between these species (Misiones region of Argentina or Paraguay) morphological patterns of geographic variation would make it difficult to separate these forms on body coloration alone. Morphometrically, these forms also overlap in multivariate space (Olds 1988, Bonvicino et al. 2003, Teta et al. 2017). *Calomys tener* is however, on average, larger (TL = 143.18 vs 132.4) and has a relatively longer tail than *C. laucha* (T/HB = 80.4 in *C. tener*, but 72.7 in *C. laucha*). *Calomys tener* has a longer maxillary toothrow than *C. laucha* (3.45 vs 3.2 mm); finally, *C. tener* can be distinguished from *C. laucha* by the presence of ridges along the sides of the supraorbital region (not present in *C. laucha*).

In a recent study, González-Ittig et al. (2014) also confounded the two species; three specimens corresponding to *C. tener* (one from Argentina and two from Paraguay) were wrongly classified as *C. laucha* according to external characters. In the present study, the same occurred with specimens from localities 16- Estancia Santa Inés (Misiones) and 17- RN 12 y Arroyo Itaembé Miní (Misiones) both in Argentina. Considering body size and cranial measurements, Salazar-Bravo et al. (2001) and Bonvicino et al. (2010) separated two groups of *Calomys*, a larger-bodied species group, including *C. callosus*, *C. expulsus*, *C. tocantinsi*, *C. callidus* and *C. cerqueirai*, and a smaller-bodied species group, including *C. tener* and *C. laucha*. However, in the phylogenetic tree obtained here (Fig. 2) and in those obtained by Almeida et al. (2007) and by Haag et al. (2007), *C. tener* is not the sister species of *C. laucha*. It is important to highlight that phylogenetic trees reported by those authors and that of Fig. 2 show the same global topology with the following relationships: (*C. tener* (*C. laucha* and the big clade including all the large-bodied species of *Calomys*)).

Quintela et al. (2014) recorded for the first time the sympatry between *C. tener* and *C. laucha* and warned that the distribution limits in the Rio Grande do Sul state in Brazil should be investigated. Considering the two clades found for *C. laucha* by González-Ittig et al. (2014), it is highly probable that what Quintela et al. (2014) found corresponds to our clade B. Thus, there is no evidence of sympatry with *C. laucha sensu stricto* (or clade A) (see a discussion on the geographic distribution of the species in Teta et al. 2017). In Rio Grande do Sul both clade B of *C. laucha* and *C. tener* could be getting in contact occasionally; it is known the two species have very low densities, because dozens of studies (and thousands of trap-nights) have been conducted throughout the state and until recently none reported captures of the two species (Cademartori et al. 2004, Badzinski et al. 2012, Quintela et al. 2012, 2014). González-Ittig et al. (2014) suggested that clade B of *C. laucha* may be experiencing a process of population expansion and dispersal into this region. The same could be occurring in *C. tener*. In the present study, many of the basal haplotypes of the Cyt-b gene are from localities of

north-central Brazil (localities 1, 4 and 5), however, the two most basal haplotypes, H_11 and H_25, are from localities 12 and 13 in Rio Grande do Sul. In the COI gene, the most basal haplotype is H_D from locality 14 also in Rio Grande do Sul; thus the results of both genes are challenging the hypothesis of a north-south colonization of *C. tener* (Figs 2, 4). Contradicting the phylogenetic trees, in the network of the Cyt-b gene, both H_11 and H_25 are in a peripheral position which is more compatible with new haplotypes (Fig. 3). Almeida et al. (2007) described an intraspecific geographic structure given that they found a clade containing samples from São Paulo state, which was clearly separated from the remaining specimens. In the present study we did not find this pattern. We found instead that some haplotype-relationships in Fig. 2 were also detected in the network (Fig. 3), but without a clear geographic pattern. Nevertheless, it is important to consider that the sample size is low and it is not possible to make too many interpretations about the demographic history of the species, what should be performed in a future phylogeographic study.

In summary, *C. tener* constitutes only one evolutionary unit corroborated by the molecular data presented here using individuals encompassing most of the known geographical distribution of the species. In the present study we tried to survey the overall genetic differentiation of the species not deepening in the genetic structure itself. Because *C. tener* is a potential human health risk by Araraquara hantavirus in South America, additional surveys in areas not represented in our sampling, coupled with a broader phylogeographic study is needed to expand our understanding of the ecological and biogeographical processes experienced by the species.

ACKNOWLEDGEMENTS

We thank the Natural Sciences Research Laboratory of the Museum of Texas Tech University, Lubbock, Texas, USA for providing tissue samples for the specimens of *C. tener*. We are very grateful to Ulyses F.J. Pardiñas (CENPAT, Puerto Madryn, Argentina), for providing most of the samples from Misiones (Argentina) used in this study. We also thank to Juan Diego Pinotti (IDEA, UNC-CONICET, Córdoba, Argentina) for his assistance to make the map. This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 2016 #1328), by the Secretaría de Ciencia y Tecnología (SECyT-UNC), Universidad Nacional de Córdoba, Argentina. We acknowledge the contribution of the Graduate School, Texas Tech University (Doctoral Dissertation Completion Fellowship to NPK) and Texas Tech University Proposal Stimulus Program (to JSB).

LITERATURE CITED

Almeida FC, Bonvicino CR, Cordeiro-Estrela P (2007) Phylogeny and Temporal diversification of *Calomys* (Rodentia, Sigmodontinae): Implications for the biogeography of an endemic genus of open/dry biomes of South America. Molecular Phylogenetics and Evolution 42(2): 449–466. https://doi.org/10.1016/j.ympev.2006.07.005

Badzinski C, Galiano D, Marinho JR (2012) Mammalia, Rodentia, Cricetidae, *Calomys laucha* (Fischer, 1814): distribution extension in southern Brazil. Check List 8(2): 264–266. https://doi.org/10.15560/8.2.264

Bonvicino CR, Almeida FC (2000) Karyotype, morphology and taxonomic status of *Calomys expulsus* (Rodentia: Sigmodontinae). Mammalia 64(3): 339–351. https://doi.org/10.1515/mamm.2000.64.3.339

Bonvicino CR, Lima JFS, Almeida FC (2003). A new species of *Calomys* Waterhouse (Rodentia, Sigmodontinae) from the Cerrado of Central Brazil. Revista Brasileira de Zoologia 20(2): 301–307. https://doi.org/10.1590/S0101-81752003000200021

Bonvicino CR, Oliveira JA, Gentile R (2010) A new species of *Calomys* (Rodentia: Sigmodontinae) from Eastern Brazil. Zootaxa 2336: 19–35. https://doi.org/10.11646/zootaxa.2336.1.2

Bordes F, Blasdell K, Morand S (2015) Transmission ecology of rodent-borne diseases: new frontiers. Integrative Zoology 10(5): 424–435. https://doi.org/10.1111/1749-4877.12149

Bradley RD, Baker RJ (2001) A test of the Genetic Species Concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82(4): 960–973. https://doi.org/10.1644/1545-1542(2001)082<0960:ATOTGS>2.0.CO;2

Cademartori CV, Fábian ME, Menegheti JO (2004) Variações na abundância de roedores (Rodentia, Sigmodontinae) em duas áreas de floresta ombrófila mista, Rio Grande do Sul, Brasil. Revista Brasileira de Zoociências 6(2): 147–167.

Cañon C, D'Elia G, Pardiñas UFJ, Lessa EP (2010) Phylogeography of *Loxodontomys micropus* with comments on the alpha taxonomy of *Loxodontomys* (Cricetidae: Sigmodontinae). Journal of Mammalogy 91(6): 1449–1458. https://doi.org/10.1644/10-MAMM-A-027.1

Case TJ, Holt RD, McPeek MA, Keitt TH (2005) The community context of species' borders: ecological and evolutionary perspectives. Oikos 108(1): 28–46. https://doi.org/10.1111/j.0030-1299.2005.13148.x

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

D'Elia G, Hurtado N, D'Anatro A (2016) Alpha taxonomy of *Dromiciops* (Micrtobiotheriidae) with the description of 2 new species of monito de monte. Journal of Mammalogy 97(4): 1136–1152. https://doi.org/10.1093/jmammal/gyw068

Dupuis JR, Roe AD, Sperling FAH (2012) Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. Molecular Ecology 21(18): 4422–4436. https://doi.org/10.1111/j.1365-294X.2012.05642.x

Fagundes V, Sato Y, Silva MJJ, Rodrigues F, Yonenaga-Yassuda Y (2001) A new species of *Calomys* (Rodentia: Sigmodontinae) from Central Brazil identified by its karyotype. Hereditas 133(3): 195–200. https://doi.org/10.1111/j.1601-5223.2000.00195.x

Figueiredo GG, Borges AA, Campos GM, Machado AM, Saggioro FP, Sabino Junior GS, Badra SJ, Ortiz AA, Figueiredo LT (2010). Diagnosis of hantavirus infection in humans and rodents in Ribeirao Preto, state of São Paulo, Brazil. Revista da Sociedade Brasileira de Medicina Tropical 43(4): 348–354. https://doi.org/10.1590/S0037-86822010000400002

Figueiredo LTM, Moreli ML, Sousa RLM, Borges AA, Figueiredo GG, Machado AM, Bisordi I, Nagasse-Suqahara TK, Suzuki A, Pereira LE, Souza RP, Souza LT, Braconi CT, Harsi CM, Andrade-Zanotto PM (2009). Hantavirus pulmonary syndrome, central plateau, southwestern, and southern Brazil. Emerging Infectious Disease 15(4): 561–567. https://doi.org/10.3201/eid1504.080289

González-Ittig RE, Patton JL, Gardenal CL (2007). Analysis of Cytochrome-b nucleotide diversity confirms a recent range expansion in *Calomys musculinus* (Rodentia, Muridae). Journal of Mammalogy 88(3): 777–783. https://doi.org/10.1644/06-MAMM-A-091R1.1

González-Ittig RE, Kandel N, Levis S, Calderon G, Salazar-Bravo J, Gardenal CN (2014). Molecular systematic of the South American rodent *Calomys laucha* (Cricetidae: Sigmodontinae), a reservoir of the Laguna Negra hantavirus. Canadian Journal of Zoology 92(12): 1093–1098. https://doi.org/10.1139/cjz-2014-0133

Guindon S, Dufayard J, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate Maximum-Likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–321. https://doi.org/10.1093/sysbio/syq010

Haag T, Muschner VC, Freitas LB, Oliveira LFO, Langguth AR, Mattevi M (2007) Phylogenetic relationships among species of the genus *Calomys* with emphasis on South American lowland taxa. Journal of Mammalogy 88(3): 769–776. https://doi.org/10.1644/05-MAMM-A-319R1.1

Hershkovitz P (1962) Evolution of Neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana, Zoology 46:1–524. https://doi.org/10.5962/bhl.title.2781

Leigh JW, Bryant D (2015) PopART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9): 1110–1116. https://doi.org/10.1111/2041-210X.12410

Linder HP, Bykova O, Dyke J, Etienne RS, Hickler T, Kühn I, Marion G, Ohlemüller R, Schymanski SJ, Singer A (2012). Biotic modifiers, environmental modulation and species distribution models. Journal of Biogeography 39 (12): 2179–2190. https://doi.org/10.1111/j.1365-2699.2012.02705.x

Mattevi MS, Haag T, Oliveira LFB, Langguth AR (2005) Chromosome characterization of Brazilian species of *Calomys* Waterhouse, 1837 from Amazon, Cerrado and Pampa domains (Rodentia, Sigmodontinae). Arquivos do Museu Nacional 63(1): 175–181.

Mills JN, Childs JE (1998) Ecological studies of rodent reservoirs: their relevance for human health. Emerging Infectious Disease 4(4): 529–537. https://doi.org/10.3201/eid0404.980403

Mills JN, Ksiazek TG, Peters CJ, Childs JE (1999) Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. Emerging Infectious Disease 5(1): 135–142. https://doi.org/10.3201/eid0501.990116

Müller L, Gonçalves GL, Cordeiro-Estrela P, Marinho JR, Althoff SL, Testoni AF, González EM, Freitas TRO. (2013) DNA barcoding of sigmodontine rodents: identifying wildlife reservoirs of zoonoses. PLoS ONE 8(11): e80282. https://doi.org/10.1371/journal.pone.0080282

Nascimento FF, Pereira LG, Geise L, Bezerra AMR, D'Andrea PS, Bonvicino CR (2011) Colonization process of the Brazilian common vesper mouse, *Calomys expulsus* (Cricetidae, Sigmodontinae): A Biogeographic hypothesis. Journal of Heredity 102(3): 260–268. https://doi.org/10.1093/jhered/esr012

Olds N (1988) A revision of the genus *Calomys* (Rodentia: Muridae). PhD thesis, New York, University of New York.

Pigot AL, Tobias JA (2013) Species interactions constrain geographic range expansion over evolutionary time. Ecology Letters 16(3): 330–338. https://doi.org/10.1111/ele.12043

Quintela FM, Santos MB, Christoff AU, Gava A (2012) Pequenos mamíferos não-voadores (Didelphimorphia, Rodentia) em dois fragmentos de matas de restinga de Rio Grande, Planície Costeira do Rio Grande do Sul. Biota Neotropica 12(1): 261–266. https://doi.org/10.1590/S1676-06032012000100021

Quintela FM, da Silveira EC, Dellagnese DG, Cademartori CV (2014) *Calomys tener* (Winge, 1887) (Rodentia: Cricetidae:-Sigmodontinae): Filling gaps. Check List 10(3): 650–654. https://doi.org/10.15560/10.3.650

Rambaut A, Drummond AJ (2007) Tracer. Oxford, University of Oxford, v. 1.5 [computer program]. Available online at http://tree.bio.ed.ac.uk/software/tracer [Accessed: 08/08/ 2018]

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Salazar-Bravo J. (2015) Genus *Calomys* Waterhouse, 1837. In: Patton JL, Pardiñas UFJ, D'Elia G (Eds) Mammals of South America. Chicago, The University of Chicago press, vol. 2, 504–505. https://doi.org/10.7208/chicago/9780226169606.001.0001

Salazar-Bravo J, Dragoo JW, Tinnin DS, Yates TL (2001) Phylogeny and evolution of the Neotropical rodent genus *Calomys*: inferences from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 20(2): 173–184. https://doi.org/10.1006/mpev.2001.0965

Simonov PS (2014) Influence of natural and anthropogenic factors on the distribution of the field mouse in the Sikhote-Alin mountains. Achievements in the Life Sciences 8(1): 85–87. https://doi.org/10.1016/j.als.2014.06.001

Soberon, J (2007) Grinnellian and Eltonian niches and geographic distribution of species. Ecology Letters 10(12): 1115–1123. https://doi.org/10.1111/j.1461-0248.2007.01107.x

Suárez-Villota EY, Quercia C, Nuñez J, Gallardo M, Himes CM, Kenagy GJ (2018) Monotypic status of the South American relictual marsupial *Dromiciops gliroides* (Microbiotheria). Journal of Mammalogy 99(4): 803–812. https://doi.org/10.1093/jmammal/gyy073

Swofford DL (1998) PAUP*: phylogenetic analysis using parsimony (* and other methods). Sunderland, Sinauer Associates Publishers, v. 4.0b10.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121

Teta P, González-Ittig RE, González EM, Pardiñas UFJ, Salazar-Bravo J (2017) Notes on the taxonomy of *Calomys laucha* (Rodentia, Cricetidae), with the designation of a neotype. Mastozoología Neotropical 24(2): 419–429.

Umetsu F, Pardini R (2007) Small mammals in a mosaic of forest remnants and anthropogenic habitats-evaluating matrix quality in an Atlantic forest landscape. Landscape Ecology 22(4): 517–530. https://doi.org/10.1007/s10980-006-9041-y

Yackulic CB, Sanderson EW, Uriarte M (2010). Anthropogenic and environmental drivers of modern range loss in large mammals. Proceedings of the National Academy of Sciences of the United State of America 108(10): 4024–4029. https://doi.org/10.1073/pnas.1015097108

Yonenaga Y (1975) Karyotypes and chromosome polymorphisms in Brazilian rodents. Caryologia (Florence) 28(2): 202–210. https://doi.org/10.1080/00087114.1975.10796617

Zeballos H, Palma RE, Marquet PA, Ceballos G (2014) Phylogenetic relationships of *Calomys sorellus* complex (Rodentia: Cricetidae), with the description of two new species. Revista Mexicana de Mastozoología (Nueva Época) 4(1): 1–23.

Submitted: October 6, 2018
Accepted: January 30, 2019
Available online: May 28, 2019
Editorial responsibility: Diego Astúa de Moraes

Author Contributions: REGI analyzed the data, wrote and revised the paper; NPK processed and analyzed the raw data in laboratory; CRB participated in original fieldwork and revised the paper; JSB conceived and designed the study and revised the paper.

Competing Interests: The authors have declared that no competing interests exist.